

What is claimed:

1. A divalent anti-T cell immunotoxin targeting moiety, comprising two monovalent antibody chains joined by a disulfide bond is at C337 of residues 228-340 within the natural IgM domain in μ CH2.
2. A divalent anti-T cell immunotoxin targeting moiety, comprising two monovalent antibody chains joined by a disulfide bond at C227 or C229, or C227 and C229, of residues 216-238 of the γ IgG hinge region, and wherein C220 is changed to P.
3. The divalent anti-T cell immunotoxin targeting moiety of claim 1, further comprising residues 447-576 in μ CH4, or residues 344-446 in μ CH3 or residues 376-346 in γ CH3 or combinations thereof.
4. The divalent anti-T cell immunotoxin targeting moiety of claim 2, further comprising residues 447-576 in μ CH4, or residues 344-446 in μ CH3 or residues 376-346 in γ CH3 or combinations thereof.
5. A divalent anti-T cell immunotoxin, comprising the targeting moiety of claims 1 and a toxin moiety, wherein the orientation of the targeting moiety to the toxin moiety is fixed so that the catalytic domain of the toxin becomes a free entity when proteolytically processed at its natural processing site under reducing conditions.

6. A divalent anti-T cell immunotoxin, comprising the targeting moiety of claims 2 and a toxin moiety, wherein the orientation of the targeting moiety to the toxin moiety is fixed so that the catalytic domain of the toxin becomes a free entity when proteolytically processed at its natural processing site under reducing conditions.

7. The divalent anti-T cell immunotoxin of claim 5, wherein the toxin moiety is diphtheria toxin, and the toxin moiety is at the amino terminus of the fusion immunotoxin.

8. The divalent anti-T cell immunotoxin of claim 6, wherein the toxin moiety is diphtheria toxin, and the toxin moiety is at the amino terminus of the fusion immunotoxin.

9. The divalent anti-T cell immunotoxin of claim 5, wherein the toxin moiety is diphtheria toxin, and the toxin moiety is at the carboxy terminus of the fusion immunotoxin, and the toxin is connected to the carboxy terminus of the antibody domain via a peptide linker containing a furin proteolytic cleavage site (RXR/KR).

10. The divalent anti-T cell immunotoxin of claim 6, wherein the toxin moiety is diphtheria toxin, and the toxin moiety is at the carboxy terminus of the fusion immunotoxin, and the toxin is connected to the carboxy terminus of the antibody domain via a peptide linker containing a furin proteolytic cleavage site (RXR/KR).

11. A divalent anti-T cell immunotoxin, comprising the targeting moiety of claim 7 and a toxin moiety, wherein the toxin moiety and the targeting moiety are thioether coupled, a single cysteine is inserted within the binding domain of the toxin moiety, and the targeting moiety has only a single free cysteine per chain.

12. A divalent anti-T cell immunotoxin, comprising the targeting moiety of claim 8 and a toxin moiety, wherein the toxin moiety and the targeting moiety are thioether coupled, a single cysteine is inserted within the binding domain of the toxin moiety, and the targeting moiety has only a single free cysteine per chain.

13. The divalent anti-T cell immunotoxin of claim 11, wherein the single cysteine projects into the solvent away from interchain contacts with the targeting moiety.

14. The divalent anti-T cell immunotoxin of claim 12, wherein the single cysteine projects into the solvent away from interchain contacts with the targeting moiety.

15. The divalent anti-T cell immunotoxin of claim 13, wherein the single cysteine of the targeting moiety used for thioether coupling is selected from the group consisting of μ CH3 C414, μ CH4 C575 and C447 of γ CH3.

16. The divalent anti-T cell immunotoxin of claim 14, wherein the single cysteine of the targeting moiety used for thioether coupling is selected from the group consisting of μ CH3 C414, μ CH4 C575 and C447 of γ CH3.

17. The divalent anti-T cell immunotoxin of claim 11, wherein the toxin moiety binding domain comprises a mutation that reduces toxin binding activity by at least 1000 fold compared to wild type toxin.

18. The divalent anti-T cell immunotoxin of claim 12, wherein the toxin moiety binding domain comprises a mutation that reduces toxin binding activity by at least 1000 fold compared to wild type toxin.

19. The divalent anti-T cell immunotoxin of claim 17, wherein the toxin moiety is full length mutant S525F (CRM9).

20. The divalent anti-T cell immunotoxin of claim 18, wherein the toxin moiety is full length mutant S525F (CRM9).

21. The divalent anti-T cell immunotoxin of claim 19, wherein the immunotoxin is a fusion protein and the sequence of domains from the amino terminus from left to right is selected from the group consisting of:

toxin moiety, μ CH2, μ CH3, VL, L, VH;
toxin moiety, μ CH2, μ CH3, μ CH4, VL, L, VH;
toxin moiety, γ CH3, H, VL, L, VH;
toxin moiety, H, VL, L, VH; and
toxin moiety, μ CH2, VL, L, VH,
toxin moiety, VL, L, VH, H, γ CH3
toxin moiety, VL, L, VH, μ CH2
toxin moiety, VL, L, VH, L, VL, L, VH

wherein L is a (G4S)₃ linker, VL and VH are the variable light and heavy domains of the anti-CD3 antibody UCHT1, and H is the γ IgG hinge.

22. The divalent anti-T cell immunotoxin of claim 20, wherein the immunotoxin is a fusion protein and the sequence of domains from the amino terminus from left to right is selected from the group consisting of:

toxin moiety, μ CH2, μ CH3, VL, L, VH;

toxin moiety, μ CH2, μ CH3, μ CH4, VL, L, VH;

toxin moiety, γ CH3, H, VL, L, VH;

toxin moiety, H, VL, L, VH; and

toxin moiety, μ CH2, VL, L, VH,

toxin moiety, VL, L, VH, H, γ CH3

toxin moiety, VL, L, VH, μ CH2

toxin moiety, VL, L, VH, L, VL, L, VH

wherein L is a (G4S)₃ linker, VL and VH are the variable light and heavy domains of the anti-CD3 antibody UCHT1, and H is the γ IgG hinge.

23. The divalent anti-T cell immunotoxin of claim 17, wherein the toxin moiety is truncated at 390 or 486.

24. The divalent anti-T cell immunotoxin of claim 18, wherein the toxin moiety is truncated at 390 or 486.

25. The divalent anti-T cell immunotoxin of claim 23, wherein the immunotoxin is a fusion protein and the sequence of domains from the amino terminus from left to right is selected from the group consisting of:

toxin moiety, μ CH2, μ CH3, VL, L, VH;

toxin moiety, μ CH2, μ CH3, μ CH4, VL, L, VH;

toxin moiety, γ CH3, H, VL, L, VH;

toxin moiety, H, VL, L, VH; and

toxin moiety, μ CH2, VL, L, VH

toxin moiety, VL, L, VH, H, γ CH3

toxin moiety, VL, L, VH, μ CH2

toxin moiety, VL, L, VH, L, VL, L, VH

wherein L is a (G4S)₃ linker, VL and VH are the variable light and heavy domains of the anti-CD3 antibody UCHT1, and H is the γ IgG hinge.

26. The divalent anti-T cell immunotoxin of claim 24, wherein the immunotoxin is a fusion protein and the sequence of domains from the amino terminus from left to right is selected from the group consisting of:

toxin moiety, μ CH2, μ CH3, VL, L, VH;

toxin moiety, μ CH2, μ CH3, μ CH4, VL, L, VH;

toxin moiety, γ CH3, H, VL, L, VH;

toxin moiety, H, VL, L, VH; and

toxin moiety, μ CH2, VL, L, VH

toxin moiety, VL, L, VH, H, γ CH3

toxin moiety, VL, L, VH, μ CH2

toxin moiety, VL, L, VH, L, VL, L, VH

wherein L is a (G4S)₃ linker, VL and VH are the variable light and heavy domains of the anti-CD3 antibody UCHT1, and H is the γ IgG hinge.

27. A method of inhibiting a rejection response by inducing immune tolerance in a recipient to foreign mammalian donor cells, comprising the steps of:

a) exposing the recipient to an immunotoxin so as to safely reduce the recipients' T-cell lymphocyte population by at least 80%; and

b) transplanting the donor cells into the recipient, such that a rejection response by the recipient to the donor organ cell is inhibited.

28. The method of claim 27, wherein the immunotoxin is the immunotoxin of claim 1.

29. The method of claim 27, wherein the immunotoxin is the immunotoxin of claim 2.

30. The method of claim 27, wherein the donor cells constitute an organ.

31. The method of claim 27, wherein the donor cells constitute tissue from an organ.

32. The method of claim 27, wherein the donor cells are allogeneic.

33. The method of claim 27, wherein the donor cells are xenogeneic.

34. The method of claim 27, further comprising administering an immunosuppressant compound to enhance the anti-T cell effects of the immunotoxin.

35. The method of claim 34, wherein the immunosuppressant compound blocks IL-12-induced induction of interferon- γ .

36. The method of claim 34, wherein the immunosuppressant compound is mycophenolate mofetil.

37. The method of claim 34, wherein the immunosuppressant compound is deoxyspergualin.

38. The method of claim 27, further comprising administering a corticosteroid.

39. The method of claim 27, wherein the immunotoxin is administered from up to several hours before to several days after the transplanting step.

40. The method of claim 34, wherein the immunosuppressant is administered beginning within about 0 to 6 hours before the transplanting step and continuing for up to several weeks after the transplantation step.

41. The method of claim 40, wherein the donor organ cell is from a cadaver.

42. The method of claim 27, further comprising administering donor bone marrow at the same time, or after, the exposure step.

Add
B3